A STUDY OF THE ORGANS OF TASTE.

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Introduction.

The small goblet-like bodies found in the superficial and lateral area of the papillæ distributed over the tongue, and sometimes upon the epiglottis, anterior pillar of the fauces and uvula, have long been recognised as the organs of taste, and are uniformly called taste-buds from this peculiar bud-like form. When they were first pointed out as the end organs of taste is uncertain, but Waller[3], in 1849, seems first to have written upon their minute structure. Weber[1], 1847, and Valentin[2], 1848, were earlier investigators upon the organs, but they performed only physiological tests, the former proving that taste sensation is greatest when the exciting substance is at the temperature of the body, and the latter likewise demonstrating that the excitement of the taste nerves depends on the concentration and not amount of solution applied. Since these investigators, a long line of observations have shown the general character of the organs to be as follows: The taste-bud is formed by cells lying with their long axis corresponding to the long axis of the bud, and arranged in the shape of a bulb or bud, the nerve fibers penetrating within and between the cells, or enveloping the bud without. The cells themselves are composed of two varieties, the fusiform or sensory cells, and the flat, sustentacular or supporting cells. The buds usually lie upon the sides and bases of the papillæ circumvallate, fungiform, or filiform; or, where such an organ exists, upon the papillæ foliatæ, for example, in the rabbit. These so-called sensory cells were early seen to possess processes extending in either direction from the body of the cell, the peripheral one reaching to the gustatory pore or vertex of the taste-bud, and the central one being early supposed to become connected with the nerve of supply.

The end organs of the body forming the organs of special sense,—sight, hearing, taste, touch and smell,—have been shown by Retzius to form two classes of organs with respect to their nerve terminations. Each sense organ may be considered as essentially constructed of a nerve cell with two processes, one making its way centrally to cluster around other nerve cells or their processes, and the other terminating peripherally. In the organ of smell the peripheral process is very short, and is directly irritated by foreign particles, being joined directly to the sensory cell of the olfactory mucous membrane. The organ of smell is then readily seen to belong to one class, while the auditory organ and organ of touch belong to the other class, where the nerve cell is found in the ganglion of the posterior spinal nerve-root, and the peripheral process, if very long, escapes in fine fibrilli among the epithelial cells forming the organ, and is acted on indirectly through the modified epithelium around which it clusters. The organs of taste were until recently referred by all authors to the first class, where the nerve was in direct continuity with the peripheral cell, which was thus essentially a nerve cell.

Recent investigators, mainly Retzius[58], Von Lenhossék[62], Arnstein[63], and Jacques[66], all writing since 1892, have denied this and striven to show that these organs belong properly to the same class as the auditory and touch organs, and thus that the nerves are nowhere in direct continuation with the cells of the bulb. Tuckerman[52], on the contrary, working a little earlier on the development of these organs, claims to have seen the sensory cells in direct connection with the nerves and, indeed, points out that the development indicates also a nervous origin for these cells themselves.

It was attracted by these variances in opinion, also because the recent observations leading to such opposite results had been done by foreign investigators, and having an unusual opportunity to get material for the study of these organs in the fetus, that I undertook investigations upon the subject of the development of the organs, and also their nervous rela-

tions. After a brief resumè, therefore, of what has been done both upon the physiology as well as anatomy of these organs, I will give an account of my own methods and results.

I.

PHYSIOLOGY OF THE ORGANS OF TASTE.

*Historical Sketch.**

Camerer[24] and Wilczynsky[34] have been able to prove satisfactorily that only parts of the tongue provided with taste-buds can give taste sensations, and according to Shore[61] a considerable area in the mid-dorsum of the tongue is, therefore, devoid of all taste-sensibility.†

Oehrwell[55] gives some valuable results bearing upon this point. He examined 125 separate papillæ (of the fusiform type) scattered over the tongue, with succinic acid, quinine and sugar.

Twenty-seven gave no response at all, indicating that they were devoid of taste-organs. Of the remaining ninety-eight, twelve reacted to succinic acid alone, three to sugar alone, while none were acted upon by quinine alone.

This would seem to give evidence that there are separate nerve fibers and endings for each fundamental sensation, but the experiments at least show that there is more than one variety of taste fibers in the majority of the papillæ.

Strong evidence of this specific difference between various nerve fibers is found in the fact that the same substance may excite a different gustatory sensation according as it is applied to the front or the back of the tongue. Thus it has been demonstrated by Howell and Kastle[49] that a certain compound of saccharin (para-brom-benzoic sulphonide) appears to most persons to be sweet when applied to the tip of the

* I do not pretend to give a complete historical sketch of this part, but only what came to my attention, while looking up the anatomy and development, and whatever may throw light upon the rest of the matter here given.

† This part of the tongue is almost entirely devoid of papillæ also.

tongue, but bitter in the region of the circumvallate papillæ. But very little seems to have been done upon the physiology of these organs, and there is ample opportunity, therefore, for much more.

ANATOMY AND DEVELOPMENT.

Historical Sketch.

Waller[3], 1849, was one of the first to study the organs of taste, doing so in the frog. He was followed by a number of others who obtained various results [see bibliography up to 1867], but microscopical technique had not been sufficiently perfected before Loven[16] and Schwalbe[17], 1867–68, pursued investigations upon these organs, and studied them simultaneously in mammals and in man. Two kinds of sensory cells were first described by these authors. One comprises the taste-cell of Loven, with which the needle-cell (stiftchenzellen) of Schwalbe is identical, and the other the staff-shaped cell (stabzellen) of Schwalbe; the latter being broader, less numerous, and less refractive.

Szabadfoldy[15] and Letzerich[18], 1868, Engelmann[19], 1868, Beale[20], 1869, Maddox[21], 1869, Von Wyss[22], 1869, Krause[23], 1870, Schulze[25], 1870, Ajtai[26], 1872, and Dittlevsen[27], 1872, did work on the organs of taste, but their results were indefinite and are best summed up by Engelmann[28], 1872, (in Stricker's Handbuch) as follows: These buds are found in the circumvallate and fungiform papillæ of all animals, and in the papillæ foliatæ of the rabbit, where they are particularly well developed. The buds lie in flask-like cavities of the epithelium which they completely fill, the wall of the cavity being formed by the epithelial cells themselves. The cells lie in very closely compressed rows around the axis of the bud, arranged like the leaves of a bud. They are composed of two kinds of cells as previously described by Loven and Schwalbe. The organs of the frog have three kinds of cells, principally, the forked cells (cells with forked processes), the broad goblet cells, and slender columnar cells.

Most of the investigations following Engelmann up to Ranvier[42], 1882, viz., Jobert[29], 1872, Klein[30], 1872, Höingschmied[31], 1873, Hoffman[33], 1875, Krause[35], 1876, Shofield[36], 1876, Lannegrâce[38], 1878, Merkel[39], 1880, Vintschgau[40], 1880, and Gottschau[41], 1882, added little of value to that previously done, all agreeing to two orders of cells in the buds—the sensory cells and supporting cells—the former being given the name of gustatory cells proper because they approach nearer to nerve elements by their form, their properties and their relation to the peripheral nerves. The only divergencies consisted in a subdivision of the second group based upon their form and the relative size of their prolongations.

Merkel[39], 1880, added besides that these supporting cells or covering cells (cellules de revetement or de soutien) were found as well in the interior as on the borders of the buds.

During this time Sertoli[32], 1874, using the method of impregnation by the gold-salts, discovered a very rich system of non-medullated nerve fibrils which spread out at the base of the papillæ. Contrary to the opinion of all the other authors, this author together with Krause[35], 1876, alone observe that the nerves do not connect directly with the epithelial elements, but describe a plexus of fibrils having such complicated terminations, that their exact mode of termination these two authors were unable to establish.

Hoffman[33], 1875, added to the observations of the others these facts, that embryonic taste-bulbs could be found in the fungiform papillæ of a four and one-half months fetus, and in the epithelium lining the epiglottis.

Klein[30], 1872, in studies upon development, made the observation that in newly born children, owing to the indistinctness of the wall in most instances, no difference is perceptible between the circumvallate and fungiform papillæ.

Ranvier[42], 1882, verified the preceding results and made an excellent study of these organs. He describes the gustatory cells as elongated spindle-shaped, with a tapering cen-

tral prolongation terminating in a homogeneous knob-like ending. They possessed an oval nucleus elongated in the longitudinal axis. The supporting cells were flatter and more irregular, with a larger nucleus, and terminating in a point at their peripheral extremity. Ranvier used the impregnation method with gold chloride, and showed the existence thereby of intra-epithelial nerve fibrils, but he believed, also, that other fibers came into direct connection with the sensory cells. He seems to have been the first also to describe the multipolar cells now recognised by all.

After Ranvier, investigations upon these organs followed two lines of work; one upon the nerves and nervous relationship of the organs, the other upon the development and genetic relationship, some authors working upon the development and others upon the nerves. In the continuation of this historical sketch the work done upon the nerves will be given first and the work upon the development of the organs afterwards.

In the study of the nerve relationship but little was added up to the time of Retzius[58], 1892. Investigations followed the results of previous writers, agreeing with them that the nerves were connected directly with the sensory cells of the bulbs and in other essentials. Drasch[44], 1883, alone observes that the direct continuity of the sensory cells with the terminations of the glosso-pharyngeal nerve he was unable to make out, but in a later article and upon further examination (Drasch[48], 1887,) he concurred entirely in the belief of direct continuity between nerves and sensory cells. According to him the glosso-pharyngeal nerve terminates as follows in the papillæ; The larger part goes to terminate in free extremities in the spaces between the epithelium of the bulb; the other fibers, sufficient in number to supply the cells, terminate directly in the gustatory cells.

The other authors who worked upon the nerve relationship were Rosenberg[46], 1886, who described the multipolar cells carefully; Schwalbe[47], 1887, who wrote now for the second time upon these organs, and Fusari et Panasci[57], 1891,

students of Golgi, who confirmed the work of previous authors in extensive observations upon these organs. Tuckerman, also, while studying the development of the organs, observes that the sensory cells are developed from the peripheral extremities of the nerve fibers, thus making these cells epiblastic in origin.

Retzius[58], 1892, and Von Lenhossék[62], 1892, followed by Arnstein[63], 1893, carried out new researches after the method of Ehrlich and Golgi, and arrived at just the opposite conclusions from the preponderance of evidence adduced by previous authors. Their conclusions were that the nerves were not continuous with the sensory cells, but ended bluntly in close proximity to them, and were distributed around and between them. Retzius and Von Lenhossék described a plexiform envelope of nerves in the form of a basket which surround the bulbs externally, and which they call peribulbar. Arnstein, for his part, has seen the cells of the bulbs individually invested and covered with nerve fibrilli applied to the surface of the cells, but never penetrating their interior, and believed that these nerves, without doubt, proceeded from the glosso-pharyngeal nerve.

Retzius and Von Lenhossék have also shown the multipolar cells, described by their predecessors, present in the sub-epithelial layer, but both deny their being of a nervous nature; Retzius, because he could not observe their connection with any nerve fibril, and Von Lenhossék, because of their morphological characters.

Jacques[66], 1893, in the latest and most exhaustive study upon these organs, particularly in regard to their nerve connections, conducted investigations upon the rabbit, dog, pig, goat, cat, sheep, rat, mouse, mole, squirrel and man. Using the method of Golgi with some special modifications, he obtained excellent impregnations to demonstrate the cells and nerves of the bulbs.

I. THE CELLS.

He found the two kinds of cells previously described, the sustentacular and the sensory cells. The former are distributed throughout the entire bud instead of being placed as enveloping cells on the exterior (so described by some authors.)

The sustentacular cells have a body occupied by a nucleus, centrally located, and two prolongations, central and peripheral. The peripheral extremity is conical and ends at the gustatory pore; the central extremity is habitually enlarged or swollen into an enlargement at the end, which frequently divides into several branches of irregular contour. Of these branches there are an irregular number, usually two; some extend to the extreme inner limit of the bulb, and others, attaining much shorter distances, are reduced to mere spines.

The gustative cells also have a body occupied by a nucleus which is oval in shape in these cells, and the position of which is very inconstant, but is more generally situated near the pore than near the base of the bulb. Agreeing with Schwalbe, the author found the peripheral expansion to be thicker than the central, and to end at the gustatory pore usually in a knob-like ending. Schwalbe had divided these cells into two varieties from the fact that some ended in a knob (stabzellen) and others in a point (stiftchenzellen), but Jacques thinks this simply a variation in the mode of ending of the same cell. The central extremity is usually single, rarely bifurcated, and ends bluntly or is enlarged into an irregular swelling at the end. In opposition to the early investigations, but agreeing with Retzius, Von Lenhossék and Arnstein, this extremity is nowhere in connection with the nerve fibrils surrounding it.

II. NERVE TERMINATIONS.

The nerves arrive at the base of the papillæ in bundles and pursue various ramifications, to the sides and top, where are given off the fibrilli usually perpendicular to the main bundles,

and which enter or surround the taste-buds by processes described below. Whether in these ramifications they actually unite in a network, or simply interlace without uniting, the author is uncertain, but he believes that the latter is true. In the distribution of these to the buds themselves the author found them pursuing three courses of supply. (1). The "fibers perigemmal" which surround the buds in an enveloping plexus, before described by Retzius and von Lenhossek; (2). The "fibers intergemmal," or those that penetrated between the buds; and (3) the "fibers intragemmal," or those entering the buds themselves, being distributed between and around the cells.

The first and second are entirely extra bulbar, only the latter penetrating within the bulbs.

The fibers intergemmal pursue a nearly straight and parallel course between the buds, and end in the vicinity of the epithelium, some terminating in small knobs, but the greater part turning upon themselves at a right angle, to continue in the bed of superficial epithelium, and end there in an oval or rounded enlargement. Many of them, however, form half or complete circuits, fish-hook forms, and various other forms. In some animals (cat) these fibers are seen to divide dichotomously, but whether they actually anastomose is uncertain.

The fibers perigemmal apply themselves closely upon the whole surface of the bud, and enveloping it altogether as a fillet, are directed towards the gustatory pore. Where the impregnation was complete, these were never seen to end at the pore, but to give off collateral branches, which often ended in knobs or points as in the first group, after circuitous courses in the epithelium about the bud.

The fibers intragemmal enter the bulb, penetrate it in various directions, and some terminate in the vicinity of the pore, after having given off collateral branches; while others turning upon themselves one or many times, terminate by swollen ends in various parts of the bud, or form minute

plexuses about a cell. In no instance, upon close observation, were any fibers found in direct communication with the extremities of the cell. Appearance of such connection always vanished after judicious and careful use of the microscope, although close apposition of the fibrilli to the cells were often apparent. No clear signs of anastomosis of these with the intergemmal or perigemmal fibers could be distinguished.

SUB-EPITHELIAL CELLS.

The existence of bipolar and multipolar cells at the base of the buds and the papillæ had been demonstrated by Ranvier[42], Rosenberg[46], Schwalbe[47], Hermann[51], Drasch[48], Retzius[58], and Von Lenhossék[62], and this author found the same thing. These were usually bipolar, rarely multipolar, possessed an oval nucleus, the two poles or prolongations being of varied length, and terminated abruptly, but in some cases were seen in actual continuation with the nerve fibers. In some cases these prolongations offered the characters of axis-cylinders and continued by a fine varicose fibril a longer or shorter course. In comparison with other nerve cells, these seem to indicate that they are of the same nature, and the author is inclined to think they are, and that thus these organs are intermediate in character between the bipolar cells of the olfactory organ, and the nerve cells of the cochlear ganglion. This hypothesis must yet be verified.

In conclusion, the author thinks that neither the sustentacular or gustative cells so-called are nerve cells, but following the hypothesis of Retzius may be called "secondary sensory cells," that is that they are simply auxiliaries to, and elements in, the production of the impression of taste upon the nerves.

Other authors, as was referred to above, studied only the development of the organs. Klein[30], 1872, made some studies upon the development and Poulton*[43], 1883, directed atten-

* Data obtained from Tuckermann.

tion to the sub-epithelial nature of the taste-bulbs, basing his conclusions on the appearance as presented in Parameles and Ornithorynchus, with the conception that the primitive terminal organ of the Ornithorynchus was replaced by one epithelial in character in the higher mammals. He also regarded the gustatory ridge of the Ornithorynchus as an intermediate form between the circumvallate and foliate types, found in higher mammals.

Lustig[45], 1884, however, made the most complete of the early studies upon the development, and studied these organs in both the rabbit and man, but failed to discover them at all in rabbit embryos. In a rabbit 120 m.m. long, that had lived thirty-six hours, the papillæ (circumvallate and foliate) bore taste-bulbs in various stages of development. In the human tongue five circumvallate papillæ were found by this author in a fetus of the fifth month, the earliest fetus he examined, but no taste-bulbs were found. In a fetus of the seventh month seven circumvallate papillæ were found and well defined taste-bulbs in each.

In a fetus of eight months the bulbs still showed their embryonic character, but bulbs existed on both the free surface and the side, those in the younger fetuses being only on the free surface. In a mature still-born child all the papillæ possessed bulbs, but they were mainly on the lateral areas [a few on the top] and differed in size, form and arrangement from the adult.

Griffini[50], 1887, and Hermann[51] both studied the development of the organs of taste, Griffini mainly by excision of the papillæ, in order to study their regeneration, and Hermann chiefly in the embryos of rabbits. Griffini noted that the bulbs first made their appearance from the sixteenth to the twentieth day after excision of the foliate papillæ of the rabbit, but not until the fortieth day in the circumvallate papillæ of the dog. The bulbs lie partly in the mucosa at first. Following section of the glosso-pharyngeal nerve, taste-bulbs begin to degenerate within twenty-three hours;

the gustatory cells first, then the supporting cells. From the seventy-sixth day after the division of the nerves, bulbs are seen in early stages of development and the axis cylinder is observed to regenerate and penetrate the epithelium, while the latter cells place themselves about the nerve fibrilli. Hermann's studies were chiefly upon the rabbit and he observed on the circumvallate papillæ of an embryo rabbit of 50 m.m. length, taste-buds in the first stages of development. The earliest forerunners of definite taste-buds he observed in the form of modified basal cells of the epithelium.* The gustatory papillæ are developed in the rabbit during the latter period of intrauterine life and in the first few days following birth.

Tuckermann[52], 1888-89, has done elaborate work upon the development of these organs in both men and animals. Nothing but a brief summary can be given here.

The papillæ foliatæ, found in many mammalia, were discovered by this author in the human tongue, reaching their highest development about birth, but appearing as early as the fourth month in the fetus.

Taste-buds were first found in a fetus of fourteen weeks† and circumvallate and fungiform papillæ (in the formative stage) first at this period. The taste-buds were distinctly subepithelial, two-thirds of the bud lying in the mucosa.

In a fetus of the fourth month five circumvallate papillæ were found, many fungiform papillæ and a few foliate papillæ in the formative stage.

In a fetus of four and one-half months, six circumvallate and numbers of fungiform and foliate papillæ were found, the latter still in the formative state. Taste-buds were found in all stages of development, some subepithelial in position and some lying partly in the mucosa and partly in the epithe-

* He would thus make them hypoblastic in origin. [AUTHOR.]

† Other authors have considered stages of development by increase in length of the fetus, as more accurate than estimations by age, a method I have been led to adopt in my own work. How this author determines the ages of his specimens he does not say.

lium. The taste-buds are all upon the free surface and none upon the sides.

In a fetus of six months, eight circumvallate were present, and numerous fungiform and foliate papillæ, the latter still only partly developed. Taste-buds are found upon all the papillæ, but are only embryonic in character upon the foliate organs. The buds occupy the sides frequently of the circumvallate and fungiform papillæ, but chiefly the upper area.

In a fetus of seven months, all the varieties of papillæ are present, and taste-buds are found upon all, but still occupy the upper areas more frequently than the lateral. Buds are found rarely upon the epiglottis and uvula. The stiftchenzellen (needle cell of Loven) and the stabzellen (staff cell of Schwalbe) are distinguishable at this stage. The sensory cells are seen to develop from the peripheral extremities of the nerve fibers, and are thus epiblastic in origin.

In a child 28 days old the circumvallate papillæ are all perfectly developed, and taste-buds are found mainly upon the lateral areas of the papillæ, rarely upon the upper surfaces. Foliate papillæ are here well developed.

In adult tongues the circumvallate papillæ vary from seven to ten in number, usually eight. Taste-buds are found entirely upon the lateral areas of the papillæ and are increased in number.

Hintze[56], 1890, also made considerable study of these organs, and worked particularly upon the human fetus. In a fetus of 50 m.m. length (referred, therefore, to the beginning of the third month) was the first appearance of a papilla, and the epithelium consisted at this time of two rows of cylindrical cells, with rounded cells lying directly upon them at the surface. Papillæ fungiform and circumvallate are both present. In an embryo, 64 m.m. in length, were the first signs of developing taste-buds, some of the cylindrical cells arranging themselves in a whirl from which develop the taste-buds*.

* This agrees with Hermann in regard to genetic relationship. [AUTHOR.]

These were found in the fungiform papillæ. In this fetus were the first signs of filiform papillæ also.

The author examined in this way nine fetuses, marking the progressive growth of the papillæ, the oldest fetus being 410 m.m. long (280 m.m. vertex to coccyx). The minute development of the buds themselves the author did not describe. The taste-buds developed first upon the free surfaces, and only as the fetuses approached full term did they appear upon the sides. No signs of karyokinesis were discovered.

Niemack[60], Rauber*[65].

Following the bibliography will be found a short list of those whom I found had worked particularly upon the taste organs in other regions than the tongue. Besides these, others who worked on the tongue have found the organs in other regions, notably Tuckermann, Gottschau, Shofield, Hönigschmied and Krause, with some others. I have not followed these out in this brief article, but only refer to them to indicate that considerable has been done on other regions than the tongue upon these organs. As was pointed out in the introduction, they have been found upon the epiglottis, the anterior pillar of the fauces and the uvula.

ORIGINAL RESEARCH.

In a study of the organs of taste two departments of investigation are open to the student: the development and the nervous anatomy. Following these two departments the present study will take up first the development of the organs in the human fetus and, secondly, the nervous anatomy.†

* Being single publications, I was unable to obtain these authors' articles.

† This work was done in the private laboratory of Prof. H. B. Ferris, of the Medical Department of Yale University, and I am much indebted to Prof. Ferris for considerable material and assistance given me.

I.

DEVELOPMENT.

In the study of the development I was able to examine sections from the tongues of twenty-four specimens*, two of which were full term. In the description of these, the best method to follow seemed to be the use of the comparative lengths rather than ages.

A reference to other authors' works, together with an examination of my own specimens, convinced me that the age, as obtained from the usual sources of information, could be little relied upon for accurate work. On the other hand, it seems to me that in the first few weeks the age can usually be approximately determined by the length of the fetus, from vertex to coccyx, or from the length of the spinal column alone. The latter seems to especially merit our favor from its necessarily constant ratio both to the size and age of the fetus, and particularly because of its ease of accurate measurement.

Minot† asserts that weight is the only available measure of the growth of the fetus as a whole. So many things, however, in the way of nutrition, change or conditions, mode of preservation, etc., cause the weight to vary, that a measurement of the spinal column would seem to be a much better indicator of the accurate age of the fetus than any other one method of determination. Inasmuch as I have received the tongue alone in some cases, with only the measurement of vertex to coccyx contributed besides, I have been unable to give here the spinal column measurement in all cases, and I have, therefore, in my descriptions, referred to the lengths as measured in toto from vertex to coccyx. This agrees also with the method of measurement employed by Hintze[56]. But

* It was owing to the kindness of New Haven physicians who have willingly responded to my request for specimens, together with the aid of Dr. Adolph Meyer, who gave me six specimens, that I have been able to give the results of work upon this number of fetuses.

† Human embryology. Minot, 1892.

I have included in each case, where possible, the length of the spinal column also, and should adopt such measurement in future work.

In order to bring to hand at once a means of estimating the age approximately from the length, I have collected statistics supplied by Minot, Lusk and other embryologists, where the age of the fetus was in each case accurately estimated from known data, and give below the lengths and age in comparative columns:

14	days	. . . . Lusk, 1.5 m.m.	length vertex to coccyx.		
23	"	. . . . His, 4 m.m.	"	"	"
27-28	"	. . . . His, Waldeyer, Müller, 7-8 m.m.	"	"	"
29-30	"	. . . . Minot, Garigues, etc., 8-10 m.m.	"	"	"
31-32	"	. . . . His, 11 m.m.	"	"	"
35	"	. . . . His, Heft, 14 m.m.	"	"	"
38	"	. . . . His, 15 m.m.	"	"	"
40	"	. . . . Minot, Waldeyer, 19-20 m.m.	"	"	"
50	"	. . . . Minot, 21 m.m.	"	"	"
60	"	. . . . Minot, 28 m.m.	"	"	"
64	"	. . . . Minot, 32 m.m.	"	"	"
75	"	. . . . Minot, 55 m.m.	"	"	"
3	months	. . . . Minot, Lusk, 80-90 m.m.	"	"	"
3½	"	. . . . Minot, 108-110 m.m.	"	"	"
4	"	. . . . Minot, 155 m.m.	"	"	"

The above cannot include variations that must occur considerably, but it gives a basis for estimate that may be relied upon for approximate determination at least.

Below is given also, in tabulated form, the comparative lengths of the fetuses examined by me, both in total lengths and length of spinal column. The length of the tongue and the approximate age of each is given also:

Fetus, No.	Length from Vertex to Coccyx.	Length of Spinal Column.	Length of Tongue.	Age (approximately).	No. of C. V. Papillæ.
1	30 m.m.	18 m.m.	. . . . .	8 weeks (2 mos.)	. . . .
2	30 "	18 "	. . . . .	8 "	. . . .
3	32 "	20 "	. . . . .	8 "	. . . .
4	60 "	"	5.5 m.m.	10 "	. . . .
5	70 "	"	6 "	10-11 "	3
6	70 "	43 "	7 "	10-11 "	4
7	80 "	49 "	7 "	12 " (3 mos.)	5
8	80 "	50 "	7 "	12 "	5
9	95 "	58 "	15 "	12-13 "	8
10	95 "	56 "	13 "	12-13 "	6
*11	100 "	63 "	13 "	14 "	7
12	115 "	75 "	14 "	14-15 "	7
13	120 "	75 "	15 "	16 " (4 mos.)	8
14	130 "	"	15 "	16 "	6
15	145 "	90 "	15 "	16-17 "	8
16	150 "	"	15 "	16-17 "	8
17	150 "	95 "	17 "	16-17 "	8
18	152 "	97 "	17 "	16-17 "	8
19	160 "	105 "	22 "	18 "	8
20	165 "	110 "	24 "	18 "	8
21	225 "	140 "	29 "	20 " (5 mos.)	8
22	230 "	150 "	30 "	20 "	8
23	300 "	200 "	34 "	25 " (6 mos.)	8
24	440 "	340 "	48 "	Full term.	8
25	450 "	340 "	50 "	Full term.	8

In studying the development of the taste organs the embryologist must necessarily direct his attention equally to the papillæ within which they appear, and compare the growth of both simultaneously. Up to the end of the second month or in fetuses of 30-40 m.m. in total length, there is very rarely any deviation from the homogeneous epithelium of the hypoblast covering the tongue. But about this period there appear definite layers of cells, consisting of a row of columnar cells which lie upon the mucosa or mesoblast below, and covering these cells the polygonal and flattened cells of the superficial layers. The columnar cells are at first parallel with the general contour of the surface, but they early show signs of assuming a sinuous course, and in fetuses of 50-80 m.m. in total length (tenth to thirteenth week) the embryonic papillæ are seen in all stages of development. (Fig. 1). It

* Not examined microscopically.

will be remembered that Hintze[56] noted the first papillæ in a fetus 50 m.m. in length.

There were three specimens which I examined, younger than the tenth week, two of which were each 31 m.m. in total length, 18 m.m., length of spinal column, and the third was 32.5 m.m. in total length, and 20 m.m. length of spinal column. In these the epithelium was homogeneous and undifferentiated.

There were also three specimens between the lengths 60 to 70 m.m., and in these papillæ were present in various grades of development. Two of these were each 70 m.m. in length, spinal column 43 m.m., and the third was 60 m.m. in length, spinal column length undetermined.

It is usually in fetuses further developed than the last, that is, in those from 80-110 m.m., that the taste-buds themselves are first seen. Hintze observed the earliest taste-bulbs in a fetus 100 m.m. in length, or in a fetus of about the fourteenth week, and Tuckerman also in one of the fourteenth week.* In my own observations there were two fetuses each of 80 m.m. length, spinal column 49 and 50 m.m. in length (about the twelfth week therefore). In both of these the papillæ circumvallate, of which there were five in both cases, were beginning to assume their characteristic form, but were below the surface epithelium, and in neither case showed any signs of taste-buds. The papillæ foliatæ were beginning to form, but were embryonic in character, and enclosed within the epithelium. The fungiform papillæ in one of the specimens were likewise only partially developed, but in the other they were well advanced in growth and their tips were elevated .05 m.m. above the surface of the surrounding epithelium. In one of these, situated on the side and at the base of the tongue, was distinctly seen a taste-bulb with but few cells, and only partially protruding into the epithelium of the papillæ in which it was growing. About half of its length lay imbedded in the sub-

* As was noted before, how Tuckerman estimated the age, either in this case or in all his work, he does not say.

epithelial connective tissue below. (Fig. 2.) This is the earliest appearance of a taste-bud that I have found recorded, and does not probably represent the average period of appearance. In another fetus, as long as 115 m.m., no signs of taste-buds were discoverable, although they probably were present. This indicates considerable variation in the time of the appearance of the buds, and inasmuch as Tuckerman[52] and Hintze[56] have both found them only from the fourteenth to the sixteenth week, and others not until the eighteenth or twentieth week, (Hoffman [33] and Lustig[45]), the fourth month would seem to be more accurately judged the average period of appearance.

The genesis of the different forms of papillæ, whether from a common early embryonic form, or each from a distinct individual origin has been discussed by Tuckerman[52], Gmelin[59] and Klein[30]; the first two asserting that the development of each form was too distinct to allow of any consideration of the origin of one form from another, while Klein urges that the embryonic papillæ are indistinguishable from one another in newly born children, and therefore of common origin. I will say, from my own observations, that as soon as the embryonic papillæ begin to form in the epithelium, while still intra-epithelial in character, they assume at once the characteristics of the papillæ which they soon become. I therefore regard them as genetically distinct in origin and development. That a gustatory area exists in the Ornithorynchus which is intermediate in form between the circumvallate and foliate types in the higher mammals is urged by Poulton[43] and might seem to indicate a development of the circumvallate from the fungiform papillæ. But the simultaneous appearance of each in the human tongue is more indicative of independent origin.

The fetuses next in size that came under my observation were two, each 95 m.m. in length, spinal column 58 m.m., and 56 m.m. respectively. In both of these specimens the circumvallate, fungiform and foliate papillæ were in the first

stages of development, though easily distinguished in character (Figs. 3 and 4). There were embryonic taste-buds to be found upon one or two of the circumvallate papillæ, of which there were eight present in one, and six in the other.

Fetus 100 m.m. in length was denuded of epithelium, so no examination could be made.

Fetus 115 m.m. in length, spinal column 75 m.m., showed no signs of taste-buds, although there were seven circumvallate papillæ present, and fungiform and foliate papillæ, all partially developed. The whole appearance of the tongue, however, was of that of an earlier stage than the size and age of the fetus would indicate.

Two fetuses, one 120 m.m. in length, spinal column 75 m.m., the other 130 m.m. total length, spinal column undetermined: The first possessed eight circumvallate papillæ, and the second, six. There was the same general appearance in both of these. The fungiform papillæ by this time are well developed and the foliate are forming rapidly, but the latter possess no taste-buds. The circumvallate and fungiform papillæ, however, have taste-buds which are largely sub-epithelial, and are developing entirely upon the free surface of the papillæ, with none at all upon the lateral areas (Fig. 3). They resemble very much in character the buds observed in the fetus of 80 m.m. length, before described, Fig. 2), and probably show the same stage of development, but occur at a more usual period for the appearance of the buds than the latter.

Fetuses from 145 to 152 m.m. in length, the measurements of these were as follows: The first, 145 m.m. total length, spinal column 90 m.m. Two were 150 m.m. total length, spinal column 95 m.m. The fourth was 152 m.m. in total length, spinal column 97 m.m. The first and last of these were tried with silver nitrate impregnation, to observe the nerve terminations, but they had evidently been dead too long before the trial was made and no results were obtained, although one of them was received six hours after birth. In

the two specimens, each 150 m.m. in length, and referred, therefore, to about the fourth month, the circumvallate, fungiform and foliate papillæ are quite well developed. Taste-buds are found on all the papillæ, here for the first time observed upon the foliate papillæ. They are present only on the upper surface of the papillæ, and are still partially sub-epithelial in position. (Figs. 5 and 6.) The foliate papillæ are thus seen to develop later and more slowly than the circumvallate or fungiform, and Tuckerman has shown that they do not reach their fullest development until the first months of childhood. In the rabbit they happen to have reached a highly specialised form, and though circumvallate papillæ are present in this animal, the foliate organs carry on the functions of taste more than the circumvallate organs do, and should be regarded probably of equal rank with the latter and not as a lower form.

It has been noticed that the taste-buds are first seen partly enclosed in the hypoblast and partly included in the mucosa below, and that in their formation the cells themselves occupy a position between both (Figs. 2, 3, 5 and 6). This would seem to indicate an origin other than hypoblastic, and Tuckerman, following other embryologists, considers that these cells originate from epiblast, and are derived, therefore, from the same source as the nerves themselves. Tuckerman describes them developing with the surrounding nerves, and the latter folding themselves about the cells as they grow. This agrees also with the discovery of Von Lenhossék* that there are cells scattered through the epidermis of the earthworm, which give off fibers that run to the central nervous system, and there like sensory fibers, fork ; one fork running headward and the other tailward within the central ganglionic chain. This leads the discoverer to the hypothesis that the special sense-cells connected with nerve fibers, as in the olfactory membrane, are true neuroblasts, in that they produce the nerve fibers connected with them. Believing, like-

* Ganglia. His Archiv., 1891.

wise, that the sense cells of the organs of taste were connected with the nerve fibers, Von Lenhossék considered them neuroblastic cells. Since further study upon the subject has changed the view of the connection of these cells with nerve fibers, the neuroblastic or epiblastic origin is no longer supported as strongly. There is no reason, however, why these cells should not be epiblastic, and be developed singly here, in the same way that the sensory cells of the retina are supposed to be developed.

On the other hand, by the conception of Retzius that the cells of the taste-buds are only "secondary sensory cells," and act simply as auxiliaries to, and elements in, the impression of taste upon the nerves, these cells may not be neuroblastic at all, but may be either hypoblastic or mesoblastic.

As was shown before, studies of Hermann and Hintze would indicate that they were hypoblastic, but since they develop from a sub-hypoblastic position they may be mesoblastic. Again, a separate origin for the sensory and supporting cells is not improbable and will be discussed later in this paper, *i. e.*, epiblastic origin for the sensory and mesoblastic or hypoblastic for the supporting cells. Such a supposition explains the fact that at their appearance the embryonic buds are partly buried in the mucosa, by the supposition that the sensory elements develop and appear first and are then wrapped around by the supporting cells of the hypoblast or mesoblast; according to Hermann, Hintze and my own observations more probably hypoblast. Until the proper staining methods can be applied to embryos at the right period for the complete study of this, the subject must remain unsettled. The cells of the early taste-buds certainly appeared in my own observations to be modified cells of the basal epithelium forming the hypoblast, but my specimens were not suitable for special staining methods and I can only speak from the use of ordinary methods.

Fetuses 160 and 165 m.m. in length, the spinal column

measurement in these being 105 m.m. and 110 m.m. respectively: All the varieties of papillæ are well developed and taste-buds are found in each, partially occupying the upper surface and partially the latter portion of the papillæ (Fig. 7). In respect to the epithelium the buds are by this time well imbedded in the epithelium and are rarely sub-epithelial in position. It is here for the first time that the taste organs are found approaching the sides of the circumvallate papillæ, a position that they occupy solely in the adult, and also in the newly-born child buds are found mainly upon the lateral surfaces. This gravitation to the sides and base of the papillæ from an original position upon the upper surface is confirmed by all authors.

Fetuses 125 m.m. and 130 m.m. in length, spinal column 140 m.m. and 150 m.m. respectively: All varieties of papillæ are present and taste-buds are seen over the entire area of the circumvallate papillæ, but with a much larger number at the top than upon the sides. In the fungiform and foliate papillæ they are also occasionally seen upon the lateral surfaces, but in the latter particularly are more frequent upon the top (Fig. 6).

The filiform papillæ described by some authors, which are more slender and somewhat longer than the fungiform or foliate, I was unable to discover earlier than in these specimens just described. Here they were present, but were embryonic in character. On the tip of the tongue no taste-buds were observed upon any of the papillæ.

Fetus 300 m.m. long and spinal column 200 m.m. long: Taste-buds are found upon the entire area of the circumvallate papillæ and upon the top chiefly of the fungiform and foliate papillæ, at the back of the tongue. On the tip of the tongue, no taste-buds at all are found upon the lateral surfaces of the papillæ found in this region, the fungiform and filiform. It is thus seen that the taste areas develop first and more rapidly at the back of the tongue and later and more slowly upon the tip of the tongue. It is needless to

say that the papillæ foliatæ and the circumvallate papillæ are found only at the back of the tongue.

Many taste-buds found at this period are well developed, are little different from those found at birth (Fig. 8), are about the same size as those at birth and contain about the same number of cells. It was noticed in the early specimens that the buds contained but few cells at first; but as the organ grows, more cells appear, until, at the maturity of the bud, there are usually from sixteen to twenty cells in each bud, as opposed to about six at first. Many of the circumvallate papillæ of six months, however, have buds still undeveloped and disposed to a large extent upon the upper area.

There are, in fact, at this age buds in most all stages of development, some even still partly sub-epithelial in character.

SUMMARY.

From the preceding it will be seen that the taste-bulbs appear generally about the fourteenth week, or in fetuses of 100 to 120 m.m. length, 60 to 75 m.m. by length of spinal column. But they may appear as early as the twelfth week, or in fetuses 80 m.m. in length, 50 m.m. in length of spinal column.

The buds appear first below the epithelium forming the mucous membrane of the tongue and gradually migrate into this, until, by the end of the sixth month, they are usually imbedded entirely within it. The epithelium of the tongue being hypoblastic in origin, this process would seem to indicate an origin other than hypoblastic for the taste cells, but they are nevertheless developed from the hypoblast, probably, as these and other observations show, unless the sensory cells alone may have a separate origin from the epiblast. More special work must be done here before it can be determined precisely.

The buds appear at first upon the upper surface of the papillæ and, in the circumvallate, gravitate, as the papillæ

develop, toward the sides and base of the papillæ, becoming at the time of birth almost entirely basal in character; in the other varieties of papillæ they occupy both the top and sides up to the time of birth, and unlike the circumvallate papillæ, which always carry taste-buds upon them, these only occasionally possess them.

The papillæ themselves begin to develop in fetuses 50 to 70 m.m. in length, 30 to 45 m.m. in length of spinal column, about the tenth week. They assume their characteristic forms at once and at the fourth month are elevated considerably above the epithelium of the mucous membrane. With the exception of the filiform they are usually fully developed by the fifth month. The fungiform papillæ seem to develop first, then the circumvallate and the foliate and lastly the filiform, which appear about the fifth month. The genesis of the different forms of papillæ are distinct and are each developed individually, no one form passing into another.

The taste areas develop earlier upon the posterior portion of the tongue than upon the anterior portion and tip of the tongue and are much more numerous upon the former.

II.

NERVE TERMINATIONS.

The subject of the nerve terminations and their relations to the cells which form the organs of taste have furnished much field for investigation and research. Because of recent contrary views upon the question of the relation of the nerve fibrilli to the sensory cells, this present paper was undertaken. After giving in some detail the methods employed for this particular study, the results will be presented.

TECHNIQUE OF THE METHODS PURSUED.

Two methods of staining the nerve fibrilli of the taste organs were used. One, the old method of Golgi, by impregnating the tissue with chromate of silver, and the

other an entirely new method of staining with methylene blue.

Golgi Method.—The silver-chromate method is the one described as Golgi's rapid method. By this method the tissue must be fresh, that is, must be used within a few hours after death. Some authors have been successful with tissues not prepared until twenty-four hours after death, but in my work I found that after six or eight hours the nerves would not receive the impregnation. The tissue tried, however, were fetuses, age about four months, and no dependence can be placed upon how long they may have been dead before expulsion from the uterus.

Either pure bichromate of potash may be employed or liquid of Müller. Small pieces of the tissue are thrown into the following mixture:

Bichromate solution, 2 to 2.5 per cent. strength . . . 8 parts.
Osmic acid, of 1 per cent. strength . . . . . 2 "

The hardening being much more rapid than with the slow method, the tissues will begin to be in a fit state for taking the silver impregnation from the second or third day. My custom was to remove from the bichromate solution after thirty-six to forty-eight hours. I seldom found, however, that the silver salt would act thoroughly at this first trial and the specimen was brought from the silver bath a second time into the bichromate solution for twenty-four to forty-eight hours, after which it was transferred a second time to the silver bath. A third time was necessary in some cases and this was usually successful, but even a fourth should be tried before failure is reported. As the tissue lies in the silver bath twenty-four to forty-eight hours, the third repeated immersion will, it will be seen, bring the tissue to about the twelfth day from its first immersion, after which time little success can be expected.

The impregnation with silver nitrate requires from twenty-four to forty-eight hours. As soon as the pieces have

attained the proper degree of hardening in the bichromate, they are brought into the bath of nitrate of silver. The usual strength of this bath is 0.75 per cent., but any strength from 0.50 to 1. per cent. may be used. Relatively large portions of the solution should be employed, at least 50 c.cm. of liquid to 1 c.cm. of tissue.

The moment the pieces of tissue are thrown into the silver bath, an abundant yellow precipitate of chromate of silver is thrown down. This, of course, weakens the solution and, if fine surface impregnation is desired, will so cover the surface epithelium that a clear picture of the final terminations is impossible. In most of the work upon nerve tissue of the central or peripheral system, this would not interfere, but in tracing the nerve fibrilli to their terminations in the superficial epithelium such a precipitate renders the view very indistinct. To prevent this, the specimen, after removal from the bichromate bath, is washed gently in distilled water and then immersed in gelatin, which has been warmed just to the melting point. The gelatin hardens at once and forms a coating around the specimen, preventing any precipitate forming when immersed in the silver and at the same time impregnation can go on as usual.

If there is no reason for using the gelatin, it is well, before putting the pieces into the final silver solution, to first wash them in a weaker solution, until, on being put into a fresh quantity no further precipitate is formed. Solutions already used will do for this purpose. This final silver bath needs little attention, except in case it should become yellow, when it should be changed for fresh.

It is not necessary to keep the preparations in the dark, but Lee says that in winter it is better to keep them in a warm place. Although the impregnation takes place within forty-eight hours, usually, "tissues may remain in the bath without hurt for days, weeks or months." (Lee's Vademecum.

In regard to preservation of the specimens, as soon as a

trial has shown sufficiently satisfactory impregnation, they are brought at once into alcohol. A trial can usually be made by making a free hand section and observing with the microscope. In this rapid method the specimens should not remain too long in alcohol, not more than two days, and are then transferred to either celloidin or paraffin. Some authors recommend the former as superior, but in my work I found both equally good, with the exception that if the celloidin block is left too long in alcohol before cutting, the impregnation is apt to wash out. With paraffin, on the contrary, I found the specimens could remain for weeks without change.

Sections being made they must be washed thoroughly in absolute alcohol, several changes, and then cleared in clove-oil, turpentine or creosote, where they should remain for ten to fifteen minutes (they may remain there for days without harm). Clove-oil was used almost altogether in my own work. They are then mounted in balsam (some authors say Damar is better, but I found no trouble with balsam), and without a cover.

I would like to repeat, in regard to successive impregnations, that in no case was I successful with the first trial at impregnation, but after repeating the immersion in both solutions for the full period, the impregnation seldom failed, although a third or a fourth was necessary in one or two cases. By this method excellent results were obtained and the investigations of recent authors verified.

Methylene Blue Method.—Methylene blue is the chloride or the zinc chloride double salt of tetramethylthionin and is distinct, therefore, from methyl blue, which is derived from diphenylamine blue. The properties of this especial form of blue were discovered by Ehrlich*, in 1885, and it has since then been used so successfully that it seems now to be effecting a revolution in histological technique. This color for staining nerve tissue must be as pure as possible and the histologist should make sure of obtaining only the pure, if

* Abh. K. Akad. Wiss. Berlin, 1885.

he will have success. The methylene blue, made by E. Merck, of Darmstadt, or by Grübler & Co., can be relied upon. The stain of the latter was used in the work done by the author.

The peculiar property of this stain in selecting the axis cylinders and the success reported by its use upon both the central nervous system and peripheral nerves led me to attempt a trial of it upon the organs of taste. All previous work upon the taste organs had been done by the use of either the silver or the gold method and it was to compare results of *this new method* with those obtained by the other that the present research was undertaken.

The method which after repeated trials finally gave me signal results is the following:

Having obtained the pure article, a solution of 1:1000 of 0.5-0.6 per cent. salt solution is made and the tissue removed from the animal that has just died is immersed at once in the solution for fifteen minutes to a half hour. Only experience can determine how long to leave a given specimen in the solution, for the stain is precarious and will reach a maximum degree of coloration in a short time, after which the nerves begin to discharge their color even more quickly than they take it up. Thus it is often found that the elements that have stained first, which are the sensory nerve fibers, will have lost most of their color by the time the remaining elements of the tissue are stained, such as muscle, connective tissue and the like. I made this mistake early in my work—*i. e.*, of leaving the specimens in the solution for hours,—thinking the thickness of the epithelium would require it, but in all cases the nerve elements were unstained. Thus the objects, if not more than a centimeter in thickness, should usually be immersed no longer than a half hour. By this method and by using specimens removed while the animal was still warm, I arrived at very satisfactory results. I regard the obtaining of the specimens as fresh, as was just stated, very important, since in several cases where I tried impregnation an hour to four hours after death no results were

obtained at all, with either weak or strong solutions of the stain. And this is rendered still more an essential feature in the method, from the fact that the intra-vitam staining by injection gives the best results.

As to the strength of the solution, the best stain was obtained with the solution 1:1000 of physiological salt solution, although strengths of 1:300, 1:500 and 1:2000 were tried for comparative results.

After the staining there are two methods of examination, one by immediate examination in a glycerin solution, after treating an hour with ammonium picrate, and the second by fixing with Bethe's solution of ammonium molybdate and mounting in paraffin or celloidin.

The first is a rapid method, but will only do for very thin specimens that do not require cutting and I found it entirely unsatisfactory in working upon these organs.

The second method, recommended by Bethe*, I found very successful. A solution is made of the following:

Ammonium molybdate	1 grm.
Distilled water	10.
Peroxide of hydrogen	1.
Hydrochloric acid	1 gtt.

On adding the peroxide a yellow color is produced and when the hydrochloric is added a white precipitate will be formed which dissolves on agitation. After removal from the stain and rinsing in salt solution, the preparations are put into the molybdic solution. This solution should not be more than a week old and is well to use cooled to zero if possible. The specimens are left in this for three to ten hours, according to size, when they are washed thoroughly in water for an hour and transferred to alcohol. After thoroughly dehydrating, two to twenty-four hours, they are cleared in xylol and imbedded in paraffin, or after clearing in xylol are transferred to alcohol, ether and celloidin. The paraffin method of imbedding I used almost entirely and found it rapid and sure.

* Archiv. f. Mik. Anat. XLIV., 1894.

RESULTS OF THE RESEARCH.

The principal results were obtained from the papillæ foliatæ of the rabbit, although successive trials were made upon the dog and the human fetus, with little or no success. The human fetuses employed were not sufficiently fresh; but why no success was obtained with the dog's tongue I was unable to determine, unless the epithelial tissue surrounding the taste organs in this animal are so impenetrable that not sufficient exposure to the stain was made.

The results upon the cells of the buds will be taken up first and the nerve terminations themselves afterwards.

The Cells.—Two kinds of cells are commonly described as forming the taste-buds, the gustative or sensory cells (Pl. II., Fig. 10) and the sustentacular or supporting cells (Pl. II., Fig. 9). Some authors, Loven[16], Schwalbe[17], principally, and others divide the gustatory cells again into two classes, the needle-shaped and staff-shaped, named thus on account of their variation in ending, the former in a point, the latter in a knob. In regard to this, it seems to me that Jacques[66] is right in thinking these to be but variations in the terminal processes of the same cell and that these cells should not be divided into distinct classes therefore (Pl. III., Fig. 4 *b*, and Pl. II., Fig. 10).

The two kinds of cells were found by both methods, staining equally well by both the Golgi and the methylene blue. The latter was found to retain its color rather longer in these cells than in the nerve fibers, the latter discharging the blue stain very readily. It was noticeable also that the gustatory or sensory cells (Figs. 1 and 2, Pl. III.) were always the first to stain with methylene blue and many sections showed only these cells and the nerve fibrilli stained. Later in the process the sustentacular cells stained, so that many views of the buds gave a picture under this stain like Fig. 2, Plate III., where the sustentacular cells were so little stained that they were hardly visible. When the sustentacular cells

were fully stained, the sensory cells and nerve fibers had frequently lost their color, while the connective and muscular tissues were all well stained. Does this indicate a possibility of common origin of the gustatory cells and nerves, and an equally common genetic relation between the sustentacular cells and connective tissue?

The peripheral endings of these cells were found to be as described by Jacques[66], the sustentacular cells ending in a conical extremity at the gustatory pore, and the gustative cells usually in a knob at the same pore (Figs. 2, 3, 4, Pl. III.)

The vital question of the connection of the central endings of the cells with the nerve fibers has been a matter of investigation and dispute, so especial attention was paid to note these extremities in the specimens stained. It will be remembered that all the old authors, up to the time of Retzius, had either boldly testified that the cells were in direct communication with the nerve fibers, or had concurred in the opinion expressed by others to that effect, with the exception of Sertoli[32] and Krause[35], who had on the contrary considered that such observations were uncertain. Drasch[44], 1883, had similarly announced the uncertainty of such direct communication, but later[48], 1887, in summing up the work done previously upon these organs, he concurred with the great number of investigators in the belief that there was immediate connection. Tuckerman[52] also, writing in 1889, observed that the communication between the gustatory cells and the nerve fibrilli was apparent early in the development of the buds.

On the other hand, Retzius[58], Von Lenhossek[62], Armstein[63] and Jacques[66] have shown, by means of special processes of nerve staining with chromate of silver and chloride of gold, that the central prolongations of the gustatory cells end bluntly in the tissue, and that the nerve fibrilli course everywhere in close apposition to the cells, but do not communicate with them.

My own observations are entirely confirmatory of the

conclusions of these later writers. The methylene blue was found to be particularly selective in staining the gustative cells, and their characters were well shown. The central extremity usually ends bluntly at the base of the bud, but may bifurcate, and then end bluntly, or in an enlarged swollen extremity (Fig. 2, Pl. III.) In no case, after close scrutiny, was there any long central process found, and never was there any connection of this extremity with a nerve fiber. The sustentacular cells did not stain with methylene blue so readily as the sensory (Fig. 3, Pl. III.) These have an enlargement usually at the terminations, which is single or may divide into two or more short branches (Fig. 4 *a*, Pl. III).

THE NERVE TERMINATIONS.

Previous authors have described the nerves as breaking up into three systems of branches upon arriving at the bud. The perigemmal, those that surround the bulb; the intergemmal, those that ramify between the bulbs; and the intragemmal, those that enter the bulb itself.

Both the external and internal were carefully observed by me, and their courses followed in many instances. In some instances the silver method had stained these better and in other instances the methylene blue was far better. In both cases the selective staining of the most minute terminations of the nerves was very satisfactory. To thus have, for comparison, the results of two entirely different methods of staining, both of which give the same conclusions, the one often forming the complement of the other, is particularly gratifying to the original investigator. Such two stains I found the silver and the blue to be.

The fibrilli of the nerves were found to ramify and form various networks, both externally and internally, but that the external should be considered to follow two distinct courses, as perigemmal and intergemmal fibers, seems superfluous, for the intergemmal are but the perigemmal interpolated between the buds.

The former are lost in the surface epithelium, probably supplying the same, similarly to the perigemmal; they terminate in the same manner and supply the same tissues. Hence, they are probably portions of the same system (Fig. 3, Pl. III).

The intragemmal fibers, on the contrary, enter the bulbs, are placed in close apposition everywhere to the cells of the buds, pursue various courses (Figs. 1 and 2, Pl. III.) and either return upon themselves or end immediately in very minute end swellings. In no case, however, did any fibers connect with or join the extremities of any of the cells. Jacques has given such an excellent description of these fibrilli and their terminations, with all of which I agree, that I will not enter into more detail, but refer to Plate III.

SUBEPITHELIAL CELLS.

The bipolar, sometimes multipolar cells, found by many authors at the bases of the buds and the papillæ, were shown somewhat distinctly by the silver method, and more distinctly by the methylene blue (Fig. 5, Pl. III.) These possess an oval nucleus, and two opposite prolongations terminating abruptly or branching, and where I was able to trace them, seemed to be continuous with the nerve fiber (Fig. 1 *b*). Jacques is inclined to think these are no other than nerve cells, they resemble them so clearly. If so, the hypothesis of Von Lenhossék, referred to before, would seem still more probable, and in such case the cells must have been actually left at the surface, though their connection with the central nervous system has remained. What office these cells perform has been a matter of conjecture. It may be they are connected with the function of taste, in the selection of certain elements for central interpretation, or it may be they have nothing to do with taste, but are cells endowed with some other function or property, possibly sensory, or possibly trophic in nature. The hypothesis of Jacques, that they are nerve cells intermediate in character between the bipolar

cells of the olfactory organ, and the nerve cells of the cochlear ganglion, must await verification.

In conclusion, every reason seems to point to the fact that all the cells of the taste-buds perform no function except as auxiliaries to the nerves in the impression of taste. Retzius thus applies the name "secondary sensory" cells to them, while Drasch considers the buds as a capillary system for absorbing a solution, and bringing it in contact with the nerves. That some selective action is carried on within the bud has been shown by physiological tests, (Oehrwell[55]) where quinine, sugar and succinic acid failed in but few cases to be selected by one and the same papillæ. That this selective action is done by the nerves is highly probable, in which case the cells forming the bud have no more than a mechanical part to perform.

Minot* calls attention to the uniformity in the specialisation of the sense-cells, in the organs of smell, sight, hearing and taste, which at once suggests that they are all derived from a common form. The similarity, he thinks, "confirms the theory that the special sense organs are modifications of ganglionic sense organs, which, in the ancestors of vertebrates, were all similar, and perhaps served a generalised function."

From the above suggestion of Minot, together with the selective action of methylene blue in staining the gustatory cells in preference to the sustentacular, the conclusion might be drawn that the former cells are entirely sensory in character, as formerly believed, and epiblastic in origin, and that the latter are mesoblastic or hypoblastic, and rightly judged as supporting cells merely. Certainly such a conclusion is worth consideration, and is not contrary to results already obtained upon the development of the organs. Between mesoblast and hypoblast for origin of the sustentacular cells, the hypoblast seems to be more probably the true origin, as was pointed out before in the discussion of the development.

* Human Embryology, 1892.

With our present knowledge, however, the conclusion would seem to be that these cells act as "secondary sensory" cells. They may all be neuroblastic in origin, or only the gustatory cells have that character, but from the relation of both to the nerve endings they would seem to take the part simply of auxiliaries or mechanical elements in the production of the sensation of taste. They would thus be in the same class with the auditory organ and the organs of touch.

It is the hope of the writer, that, since methylene blue has now been shown to be so successful in nerve staining by investigators generally, its use will be carried into many other investigations, upon these organs, and so decide the few points still unsettled concerning their function and genetic relations.

The object of this paper has been to present the results of previous investigations, together with such facts as my own work has accomplished, in as brief a form as possible.

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PLATE I.

Fig. 1. Vertical section of epithelium of tongue of human fetus at stage when the papillæ are just beginning to form (tenth to twelfth week of fetal life); *a*, columnar epithelium; *b*, polygonal intermediate epithelium; *c*, flattened surface epithelium; *d*, cells of connective tissue of the mucosa.

Fig. 2. Vertical section through a fungiform papilla of a fetus about the twelfth week; *a*, columnar epithelium; *b*, polygonal intermediate epithelium; *c*, flattened surface epithelium; *d*, cells of the connective tissue of the mucosa; *t*, embryonic taste-bud, consisting of but few cells, and one-half subepithelial in position. The papilla itself is only partially developed, the trench on one side being filled with epithelial cells.

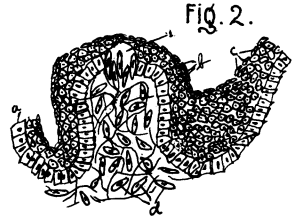
Fig. 3. Vertical section through a partially developed circumvallate papilla of fetus of the twelfth to fourteenth week. The papilla is still on a level with the epithelium, and the trenches or valleys on the side are closed.

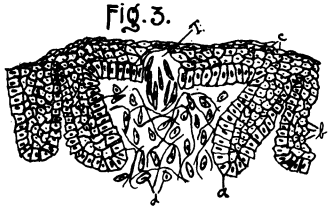
Fig. 4. Vertical section through several foliate papillæ of about the same age as the preceding, showing their embryonic character, trenches still filled, and no taste-buds; *a*, *b*, *c*, *d*, same as above.

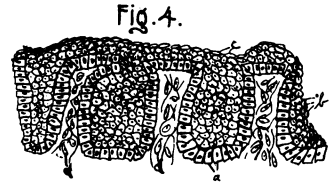
Fig. 5. Vertical section of circumvallate papilla of fetus of about the fourth month, showing the valley well formed on the sides, and the taste-buds further developed, and imbedded more deeply in the epithelium; *t*, taste-bud.

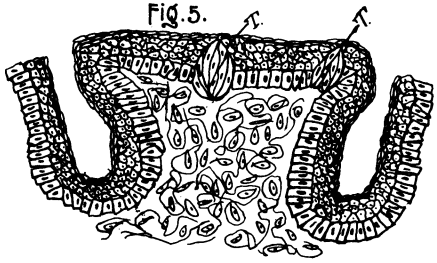
Fig. 6. Vertical section through a foliate papilla of fetus of about the fourth month, showing trenches partially developed, and an embryonic taste-bud partially concealed in the epithelium.

PLATE I.









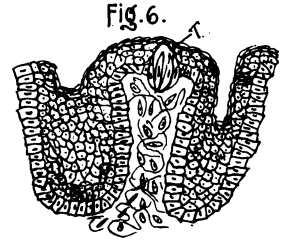


PLATE II.

Fig. 7. Vertical section through a circumvallate papilla of fetus of about the fifth month of intrauterine life, showing the taste-buds entirely imbedded in the superficial epithelium, and gravitating towards the sides and base of the papilla. The papilla itself is well developed.

Fig. 8. Vertical section through the base and side of a circumvallate papilla of fetus of about the sixth month, exhibiting only the lower portion of the sides. Here the buds have become mature, very little different from the adult, and occupy the base and sides of the papilla almost exclusively. Are rarely found on top.

Fig. 9. Characteristic supporting or sustentacular cells of the taste-bulbs of fetus near birth, the bulbs being well developed ; *b*, central end ; *a*, peripheral end.

Fig. 10. Characteristic sensory or gustatory cells of taste-bulbs of fetus near birth, the bulbs being well developed ; *b*, central end ; *a*, peripheral end.

PLATE II.

Fig 7.

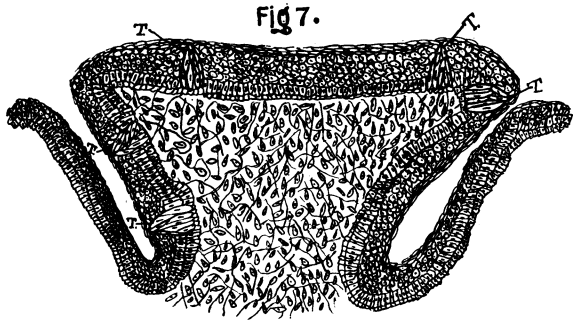


Fig. 8.

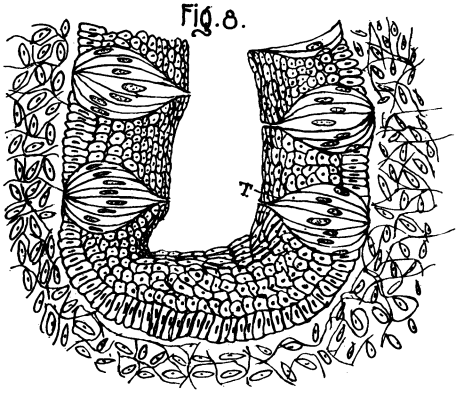


Fig. 9.

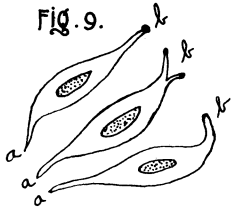


Fig. 10.

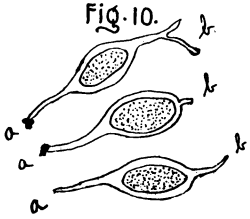


PLATE III.

Fig. 1. Vertical section through a papilla foliata of the rabbit, showing the distribution of the nerves to the surface and sides of the papilla ; *a*, perigemmal and intergemmal nerve fibrilli ; *b*, multipolar and bipolar cells of the tissue, surrounding and at the base of the taste-buds ; *c*, gustatory cells of the buds, the sustentacular cells not being stained ; *d*, the chief nerve trunk supplying the papilla, and branching to go to the buds.

Fig. 2. Vertical section through a taste-bud, where the gustatory cells only are stained, together with the nerve fibrilli, and their relation to one another is shown.

Fig. 3. Diagrammatic section through a taste-bud where the sustentacular cells only are shown, and the distribution of the external nerve fibrilli (peri- and intergemmal fibers of Jacques) in relation to these cells is represented.

Fig. 4. *a*, typical sustentacular cells as stained with methylene blue, showing different manner of ending 1 and 2 ; *b*, typical gustatory cells as stained with methylene blue, showing the different manner of ending, 1 and 2.

Fig. 5. Bipolar or multipolar cells found at the bases of the papillæ and the taste-buds, and usually connected with the nerve fibers.

PLATE III.

Fig. 1.

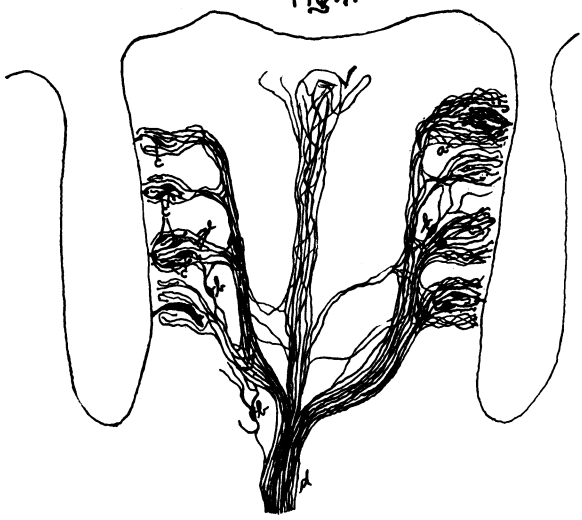


Fig. 2.

Fig. 3.

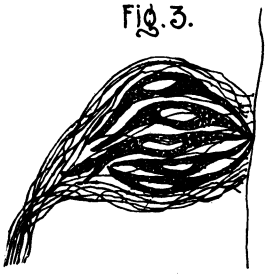


Fig. 4.

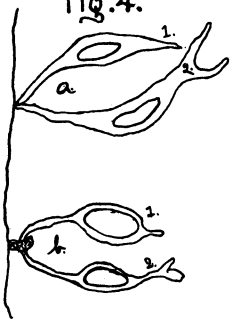
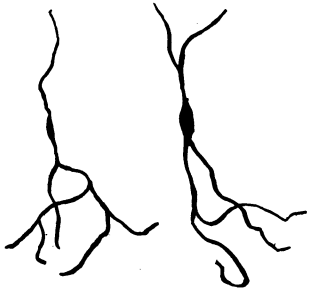


Fig. 5.



It would be unjust to overlook the fact that there are workers with the microscope whose interest in problems is not that of the biologist. From the nature of the subject, however, from its distant yet direct relation to our own existence, from the abundance of living things which surround us on every hand, and no less from the intensity of interest which accompanies the manifestation of life, those students who employ the microscope as an instrument in biological investigation, whether it be that of the specialist, or of the chance student of nature, always have and always will far outnumber those who regard the microscope from all other standpoints. It is for these, then, the students of living things, and from their standpoint that microscopical methods should be judged rather than from the standpoint of any much more limited group of other students.

There always will be a few physicists whose studies will be largely devoted to the improvement of the optical qualities of the instrument. Great advances have really been made in the past few years by the studies of specialists in this particular line, and we may well look to the future for further important advances in this respect, but the province of the biologist hardly includes such questions. The microscope is to him purely an instrument. He takes it in the shape in which it is furnished to him, demands that it shall be capable of performing the best work in the simplest manner, and employs it as a valuable tool for the furtherance of his researches. It seems to me that the failure of microscopic study from the optical standpoint, simply, or even largely, may be well illustrated by a single instance: The diatomists are not even yet agreed as to the interpretation which shall be placed upon a direct image, which is presented by the microscope. Apparently under present conditions, the application of purely physical methods to the solution of biological problems has reached its limit. Other tendencies have taken its place which have yielded fruitful results and the future, it is clear, bids fair to accentuate further the tenden-

cies away from mere study of the instrument as an optical combination.

The older microscopists, of whom I have been speaking, took the objects of their study with little or no previous preparation, except it was, in some cases, such as to remove the living matter and leave test or skeleton in condition for examination.

The first students of the more modern biological school, on the other hand, were not content with this consideration of mere dry bones, but bent their energies toward the more careful study of the living matter itself. Here they came at first face to face with one of the most characteristic features of that living substance, its changeability, and watching from moment to moment the modifications which arose in its consistency, they were anxious not only to fix it in its actual condition at a moment of time, that the details might be more carefully examined, but further to render it transparent and thus get a glimpse of the processes which were being carried on within it, processes altogether too dimly outlined through the changing and commonly decidedly opaque substance of the living cell. Methods of killing and mounting were indeed known, but now their reliability came to be tested and the first question was, how accurately do they present conditions which actually exist within the interior? and, secondly, how may the object thus reliably fixed be further treated so as to present it in the condition most favorable for study? Along this line grew up gradually, yet withal rapidly, an extensive technique, which marks the second tendency in methods of microscopical investigation. Methods of hardening, fixing, staining and, most valuable of all, of sectioning, have been evolved and tested by the most crucial experiments which eager workers could devise, and yet the advance here has not been simply an increase in the number and kinds of methods. These processes have yielded abundant fruit; the wonderful researches of the last quarter of a century have given an insight into biological problems

which has far surpassed the hopes of the most sanguine. Problems of the cell, so intimately and fundamentally connected with all biological science, are pressing towards their solution under the assistance given by these methods. It is also clear that we have by no means reached the limit of this tendency in microscopical technique. Every month, almost every week, brings new results attained by the modification or more careful application of these methods and by the introduction of new means of preparation.

To the student who examines carefully the course of the past and the needs of the present, there is, however, at least one direction in which future investigation is bound to build a new road for itself and to advance along a new line. The methods in vogue today for the examination and study of living substance are but little improved over those which obtained some thirty years ago; if possible we can see a little more, it is because we have better lenses and better instruments. The cell as a living thing, as regards the changes which take place during its processes, is known by inference from the dead object rather than by observations upon its living substance. It is a chemical laboratory and should be studied that we may know the reactions which are taking place in it. If the methods of microscopical technique most generally in vogue at the present have given us, as it were, a series of instantaneous photographs of the cell and of the arrangement or rearrangement of its various parts in various conditions, there yet remains to be developed that technique which shall show us these substances in the process of synthesis and analysis, that the investigator may be able to follow the workings of the cell as a formative power and come thus one step nearer the solution of the question, How does living matter operate?